Claims:

1. A method for determining the presence of a nucleic acid in a sample comprising the steps of

providing a fluorescent entity capable of indicating the presence of the nucleic acid and capable of providing a signal related to the quantity of the nucleic acid,

amplifying the nucleic acid through a plurality of amplification cycles in the presence of the fluorescent entity,

measuring fluorescence intensity of the fluorescent entity at each of the plurality of amplification cycles to produce a fluorescent value for each cycle related to the quantity of the nucleic acid present at each cycle,

generating a plot wherein the fluorescent values are recorded for each amplification cycle,

performing a confidence band analysis on the plot to generate a positive or negative call, and

if the call is positive, confirming the positive call by a melting temperature analysis.

2. The method of claim 1 wherein the confidence band analysis is performed by

calculating slopes of segments of the plot using a plurality of the fluorescent values,

using the segment slopes of the plot to establish a baseline fluorescence region by generating a slope value for each of a plurality of the amplification cycles, and establishing the baseline fluorescence region comprising an interval of cycles that includes the amplification cycle with the slope value having an absolute value closest to zero, and

making the positive or negative call based on whether the fluorescence value during a selected amplification cycle is outside the baseline fluorescence region.

3. The method of claim 2 wherein the baseline fluorescent region is established without the use of an internal standard.

4. The method of claim 1 wherein the melting temperature analysis is performed by

obtaining a melting profile,

determining the minimum or maximum of the first derivative to generate a Tm value, and

comparing the Tm value with the known Tm of the target analyte.

5. The method of claim 4 wherein the melting profile is obtained by monitoring fluorescence between extension and denaturation during one of the amplification cycles.

6. The method of claim 4 wherein the melting profile is obtained by monitoring fluorescence between annealing and denaturation during one of the amplification cycles.

7. The method of claim 4 wherein the melting profile is obtained by monitoring fluorescence in a separate melting process subsequent to amplification.

8. The method of claim 4 wherein the melting profile is obtained by monitoring fluorescence at 0.1°C temperature increments.

9. The method of claim 4 wherein the melting profile is obtained by monitoring fluorescence at temperature increments of greater than 0.1°C.

10. An automated method for determining the presence of a nucleic acid comprising the steps of

placing a sample into a container containing a fluorescent entity capable of indicating the presence of the nucleic acid and capable of providing a signal related to the quantity of the nucleic acid,

placing the container into a device for amplifying the nucleic acid through a plurality of amplification cycles in the presence of the fluorescent entity,

measuring fluorescence intensity of the fluorescent entity at each of the plurality of amplification cycles to produce a fluorescent value for each cycle related to the quantity of the nucleic acid present at each cycle,

generating a plot wherein the fluorescent values are recorded for each amplification cycle,

calculating slopes of segments of the plot using a plurality of the fluorescent values,

using the segment slopes of the plot to establish a baseline fluorescence region by generating a slope value for each of a plurality of the amplification cycles, and establishing the baseline fluorescence region comprising an interval of cycles that includes the amplification cycle with the slope value having an absolute value closest to zero,

outputting a positive result if the fluorescence value of a selected amplification cycle is outside the baseline fluorescence region, and

confirming the positive result by melting temperature analysis.

11. A device for determining the presence of a predetermined nucleic acid in a sample comprising

an instrument for temperature cycling to amplify the nucleic acid,

a fluorimeter for detecting fluorescence during amplification of the nucleic acid, the fluorescence obtained from a fluorescent entity capable of providing a signal related to the quantity of the nucleic acid, wherein the fluorimeter measures fluorescence intensity of the fluorescent entity at each of a plurality of amplification cycles, and wherein the fluorimeter measures fluorescence intensity of the fluorescent entity to obtain a melting profile of the nucleic acid, and

a processor for performing analysis routines, wherein the processor is programmed to generate a plot of the fluorescent values verses amplification cycle, to perform a confidence band analysis on the plot to generate a positive or negative call, and to perform melting temperature analysis to confirm a positive call.

12. The device of claim 11 wherein the instrument is configured for rapid thermal cycling.

13. The device of claim 12 wherein the instrument employs capillary tubes and hot air control.